THE INFLUENCE OF SOME AMINO-ACIDS ON THE DEVELOPMENT OF ECHINODERMS.[1]

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While a great deal has been done on the influence of various external factors on development, little is known concerning the influence of those various metabolic products produced by the organism itself. It is probable that such products influence both the rate and character of development and it has long seemed to me that by varying them we could most surely reach our goal of an understanding and control of the mechanism of development. Accordingly I added to sea-water containing developing eggs of the sea-urchin, *Arbacia punctulata*, various amounts of cystin, leucin and tyrosin to see what changes would be produced in development.

The most interesting result was obtained with cystin. Cystin is the sulfur-containing amino-acid of the albumin molecule and has the composition, $C_6S_2N_2H_{12}O_4$. The sample I used was obtained by Mörner's method from horn. It is almost insoluble in cold water. One hundred cubic centimeters of sea-water were shaken for a moment with about a centigram of crystalline cystin and the mixture poured into a finger bowl with the undissolved cystin. The eggs, fertilized something less than an hour before, the time varying in different experiments, were then added and the eggs lay during development among the crystals of cystin at the bottom of the dish. At the same time similar transfers from the same lot of eggs were made to sea-water shaken in the same way but without the cystin. This was to throw out the possibility that aeration or some other factor was responsible for the results obtained.

The sea-urchin eggs this summer showed again, in many instances, the remarkable peculiarity recorded by Whitcher[2] and myself, a large number of eggs while living for several days not forming plutei, or but a small per cent. of irregular plutei, and the proportion of these going abnormally was increased by the

[1] From the Marine Biological Laboratory, Woods Holl.

[2] Mathews and Whitcher, *Amer. Journal of Physiol.*, VIII., 1903, p. 300.

act of transfer. The effect of cystin on such eggs was indeed most striking. Whereas in the controls hardly a pluteus was to be found and these few were generally abnormal, the cystin eggs nearly all formed normal plutei. The effect, also, of the cystin on the development of the more normal eggs was perfectly invariable. The cystin eggs always showed a decided acceleration of development, so that they were plutei while the controls were still gastrulæ. The acceleration was visible from about the fourth division on. Plutei formed in the cystin solutions within 24 hours of fertilization. The cystin not only accelerates the process of pluteus formation, but a gradual acceleration of development takes place from the start.

We have, therefore, in cystin a substance which acts most beneficially on *Arbacia* development, particularly in those cases where the development is for some reason abnormal. It resembles in this particular the action of alkalies as recorded by Loeb[1] and pilocarpine in the case of star-fish eggs.[2] The conclusion is not of course justified that the abnormality is due to a deficiency of cystin possibly produced normally by intracellular digestion, although such a possibility is not in itself improbable. If the eggs are left in contact with cystin their death takes place after 48 hours, but if they are transferred to fresh sea-water after 24 hours in the cystin water, their subsequent development does not appear to be interfered with. They develop normally thereafter.

Toward the star-fish eggs cystin proved to be toxic and the only effect noticed was the early death of the egg. No acceleration was produced in the development of the mollusc, *Cumingia*, by the addition of cystin. The action appears, therefore, to be rather specific for *Arbacia*.

I also tried the action of tyrosin and an impure leucin from horn upon *Arbacia* eggs. The tyrosin, while not very soluble, is more soluble than cystin and accordingly more of it is in solution to act on the eggs. A saturated solution was at first used, the sea-water always containing undissolved tyrosin crystals. The effect of such a saturated solution on the development of *Arbacia*, when the experiments were tried in the same way as those with cystin, was invariably harmful; development was retarded and the eggs

[1] Loeb, *Archiv für Entwickelungsmechanik*, 1898, VII., p. 631.

[2] Mathews, *Amer. Journal of Physiol.*, VI., 1901, p. 207.

ultimately killed. With weaker solutions the results were uncertain. In some cases the development was slightly accelerated, but more often it was either not affected or else retarded.

Impure leucin prepared from horn proved very toxic, possibly owing to some impurity, but possibly to the leucin itself. The effect of a weak solution is extremely interesting in that it arrested development without killing the eggs. Particularly it prevented development beyond the blastula stage. The embryos were unable to escape from the membranes; they remained without farther development in these membranes but still alive, for 24 to 72 hours. The color gradually disappeared until they were as colorless as star-fish larvæ. I took some of them out to fresh sea-water after being thus blastulæ for 30 to 40 hours, to see whether their subsequent development would be interfered with. After a time the embryos emerged from the membranes and swam about; they lived for days and developed into all sorts of fantastic embryos. Some of them were totally unlike *Arbacia* larvæ. In many, evagination of the entoderm instead of invagination, took place. A few developed a ciliated band in the shape of the star-fish *bipinnaria* larvæ which they resembled far more nearly than they did *Arbacia*. These forms, however, never developed a well marked entoderm like that of the star-fish, nor did the mouth and anus invaginate. Another highly interesting form was perfectly spherical with a single ciliated band about the middle and swimming rapidly. It looked in its external form like a very small trochophore. Obviously the sample of leucin which was used checked some particular development but allowed other processes to go on so that the embryos lived but developed in a widely different direction from normal. I know of no means of proving so clearly the infinite number of different embryonic forms resident in each egg. Possibly an examination of these different forms might be of decided interest, and I hope that some embryologist may think the subject worth investigating since it lies apart from my own field. I was unable to make all the embryos abnormal in one direction by leucin, but I did not particularly investigate this possibility.

These experiments show that the products of intracellular protein digestion may be very important in determining development.